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U.S. Patent Application For

HIGH-EFFICIENCY MICROARRAY PRINTING DEVICE

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HIGH-EFFICIENCY MICROARRAY PRINTING DEVICE

CROSS-REFERENCE TO RELATED APPLICATIONS

[Not Applicable]

STATEMENT AS TO RIGHTS TO INVENTIONS MADE UNDER FEDERALLY SPONSORED RESEARCH AND DEVELOPMENT

[Not Applicable]

FIELD OF THE INVENTION

[0001] This invention pertains to the field of high-density microarray production. In particular, this invention provides methods and devices that permit high-density arrays to be printed with significantly smaller feature size and spacing and greatly improved reagent usage.

BACKGROUND OF THE INVENTION

[0002] The immobilization of test molecules or "probes" on array supports has had a significant impact on drug discovery, medical diagnostic methods, and basic research. The use of high-density microarrays of organic molecules permits literally thousands of assays to be simultaneously performed on one or more samples. Using high-density microarrays, numerous analytes can be simultaneously detected and/or quantified permitting the rapid characterization of complex systems (*e.g.* complex assays for gene expression). High-density microarrays are also useful for "high-throughput" screening assays, diagnostics, and in many other contexts. The ability to manufacture microarrays in an efficient and cost-effective manner is of considerable interest to researchers worldwide and of significant commercial value.

[0003] In general, microarrays of greater density are preferred. A higher density array typically allows more assays to be performed simultaneously and/or, for lower sample volumes to be used for the same number of assays. In providing large, high-density arrays of molecules (*e.g.*, probes or analytes) there are a number of considerations. The array elements (*e.g.* dots) should be substantially reproducible in size, particularly if one wishes to quantify an analyte.

In addition, the array elements should be consistently and reliably positioned, and should be highly reproducible.

[0004] The basic approaches for generating arrays of test molecules such as nucleic acid, protein or other organic molecules fall into two general categories. In the first such approach the test molecules are directly synthesized onto the array support, while in the second such approach the test molecules are attached to the support post-synthetically. Each approach has its own limitations and drawbacks. For example, when an array is created by direct synthesis onto an array support, the efficiency of each synthetic step affects the quality and integrity of molecules forming the array. The magnitude of the problem increases with the complexity of the individual molecules, potentially resulting in an undesirable percentage of incorrectly synthesized molecules and incomplete sequences. Such contaminants can interfere with subsequent use of the array.

[0005] In addition, synthetic approaches (*e.g.* as described by Southern *et al.* (U.S. Pat. Nos. 5,770,367, 5,700,637, and 5,436,327), Pirrung *et al.* (U.S. Pat. No. 5,143,854), Fodor *et al.* (U.S. Pat. Nos. 5,744,305 and 5,800,992), and Winkler *et al.* (U.S. Pat. No. 5,384,261), are generally unable to construct microarrays of large macromolecules. Such technologies can also be expensive and difficult to implement.

[0006] In contrast, the second approach to array production allows the desired molecules to be synthesized and purified by conventional methods prior to their formation into an array. Consequently, the quality of the arrayed molecules, and thus the quality of the resultant array, is potentially greater than that produced by the direct synthesis approach.

[0007] Such "spotting" approaches include, but are not limited to inkjet, and direct surface contact printing. Inkjet devices require high reagent volumes and risk "probe" degradation during volatilization.

[0008] Direct surface contact printing (*see, e.g.*, U.S. Patents 4,981,783, 5,525,464, 5,770,151, and 5,807,522), are limited in their ability to reliably, reproducibly, and uniformly apply the array elements to the array substrate. Reagent usage is also relatively inefficient, and array density is limited.

SUMMARY OF THE INVENTION

[0009] The present invention provides improved components (*e.g.* array "pins", print head, substrate platen, print head platen, and the like) for microarray printing devices as well as microarray printing devices incorporating such components. In particular, methods and devices of this invention permit high-density arrays to be printed with significantly smaller feature size and spacing, and greatly improved reagent usage.

[0010] In one embodiment this invention provides a microarray print head, said print head comprising a plurality of glass or quartz (or other mineral), or ceramic, or porcelain, or ceramic spotting capillaries disposed in a support that maintains a fixed spacing between the spotting capillaries and that permits the spotting capillaries to move in a direction parallel to the long axis of the capillaries (*i.e.* the spotting capillaries can slide through the support). Preferred spotting capillaries are microcapillary tubes and particularly preferred spotting capillaries have a tapered tip (*e.g.* a ground, beveled tip). The capillaries can have any desirable cross-section (*e.g.* round, ovoid, square, triangular, irregular), however preferred capillaries are round in cross-section.

[0011] In certain preferred embodiments, the capillaries have a maximum load volume of about 0.5 mL. In certain preferred embodiments, the spotting capillaries have a load volume of about 0.2 mL.

[0012] Preferred print heads comprise at least 4 spotting capillaries, preferably least , 4, 16, 32, 64, or 128 spotting capillaries and in certain preferred embodiments, the spacing between two adjacent spotting capillaries is about 3 mm or less, center to center.

[0013] In certain preferred embodiments, the spotting capillaries have detents where the spotting capillaries have a rest position in which the detents contact a support stopping the movement of the spotting capillaries in a direction toward the substrate that is to be printed. The print head can also comprise a spring attached to a spotting capillary where, in the absence of a force against the printing tip of the spotting capillary the spring returns said spotting capillary to a rest position. The print head can be provided separately or can be found as a component in a microarray printing device.

[0014] In preferred embodiments, the spotting capillaries are in fluid communication (e.g. via flexible capillary tubing) with a manifold. In preferred embodiments, the manifold comprises a common port and a plurality of individual ports where an aperture into an individual port is disposed inward of the inside wall of the manifold. The manifold can be
5 connected to a gas and/or vacuum source.

[0015] In another embodiment this invention provides a platen for positioning a substrate holder or a print head in a microarray printing device. A preferred platen comprises a support surface attached to a single guide rail such that the support surface can move along the guide rail, and motion of the support is constrained in a direction normal to the guide rail, and a
10 flexible coupling to an actuator wherein the flexible coupling is rigid or stiff in a direction parallel to the guide rail, but is flexible in another direction. The platen also, optionally, comprises an encoder (e.g. optical encoder, magnetic encoder, electronic encoder, *etc.*) that encodes the position of said platen along said guide rail. In certain embodiments, the platen is attached to the rail by two bearings. Preferred flexible couplings include, but are not limited to
15 a flexible sheet coupling (e.g. sheet metal, sheet plastic, *etc.*), a rod bearing, a ball bearing, a pin bearing and the like. Preferred actuators include, but are not limited to a stepping motor, a linear motor, a lead screw, and the like. In certain embodiments, the platen can further comprise a holder (e.g. a slide holder) for one or more microarray substrates. In certain embodiments, the platen is attached to a microarray print head (e.g. directly or through a movable stage). Preferred print heads in such cases include, but are not limited to any of the
20 print heads described herein.

[0016] In still another embodiment, this invention provides a microarray printing device comprising a microarray print head (e.g. as described herein); and a microarray substrate holder attached to a platen (e.g. a platen as described herein). Preferred microarray printers can print
25 at least 2,000, more preferably at least 5,000 array elements per spotting capillary per load. Preferred microarray printers can print array elements with a precision of at least 30 μm and/or with an average inter-element spacing of 130 μm or less. Preferred microarray printers can print 200 or more microarray substrates in a run. Particularly preferred microarray printers of this invention utilize pressure and/or vacuum to control reagent loading or dispensing. Certain
30 microarray printers comprise the spotting capillaries are in fluid communication with a

manifold. A preferred manifold comprises a common port and individual ports where an aperture into an individual port is disposed inward of the inside wall of the manifold. In certain preferred embodiments, the microarray printing device can load reagents from a microtiter plate comprising at least about 864 wells.

5 [0017] This invention also provides a method of printing a microarray (*e.g.*, a nucleic acid and/or protein and/or small organic molecule microarray). The methods involve providing an array substrate in a microarray printing device comprising one or more of the elements (*e.g.* spotting capillaries, print head, array substrate platen, print head platen, and the like) as described herein, providing a series of solutions comprising the reagents that will form features
10 of the microarray; and operating said microarray printing device to print the microarray. In preferred methods the microarray printing device prints a microarray comprising at least 1,000 different array elements. In preferred methods the microarray printing device prints a microarray comprising having an average inter-feature spacing of about 130 μm or less. Preferred array substrates include, glass, quartz or other minerals, metals, ceramics, plastics, metal coated glass, metal coated plastic and the like. In preferred methods the microarray
15 printing device applies negative pressure to load a spotting capillary and/or positive pressure to dispense from a spotting capillary. In preferred embodiments, the method involves loading feature-forming reagents from a microtiter plate comprising at least about 864 wells.

[0018] In still another embodiment, this invention provides (printed) microarrays.
20 Preferred printed microarrays comprise at least about 1000 different array elements on an array substrate, where the array elements are separated by an average center to center spacing of about 130 μm or less, preferably about 100 μm or less, more preferably about 80 μm or less. Where the arrays are nucleic acid and/or protein arrays, the protein or said nucleic acid is preferably not a chemically synthesized protein or nucleic acid. Particularly preferred
25 microarrays include nucleic acid nucleic acid microarrays. In certain embodiments, the nucleic acids comprising such microarrays have an average length greater than about 50, preferably greater than about 100, 200, or 500 nucleotides, more preferably greater than about 1000 nucleotides. The molecules comprising the array features are preferably adsorbed to the array substrate. In certain nucleic acid or protein arrays the nucleic acid or protein is not covalently
30 coupled to the array substrate (*e.g.* not coupled directly or through a linker to a terminal

nucleotide or amino acid). In particularly preferred microarrays, the features comprising the arrays are at an average density of about 40,000/cm² or greater.

DEFINITIONS

[0019] The terms "polypeptide", "peptide" and "protein" are used interchangeably herein to refer to a polymer of amino acid residues. The terms apply to amino acid polymers in which one or more amino acid residue is an artificial chemical analogue of a corresponding naturally occurring amino acid, as well as to naturally occurring amino acid polymers. The term also includes variants on the traditional peptide linkage joining the amino acids making up the polypeptide.

[0020] The terms "nucleic acid" or "oligonucleotide" or grammatical equivalents herein refer to at least two nucleotides covalently linked together. A nucleic acid of the present invention is preferably single-stranded or double stranded and will generally contain phosphodiester bonds, although in some cases, as outlined below, nucleic acid analogs are included that may have alternate backbones, comprising, for example, phosphoramidate (Beaucage *et al.* (1993) *Tetrahedron* 49(10):1925) and references therein; Letsinger (1970) *J. Org. Chem.* 35:3800; Sprinzl *et al.* (1977) *Eur. J. Biochem.* 81: 579; Letsinger *et al.* (1986) *Nucl. Acids Res.* 14: 3487; Sawai *et al.* (1984) *Chem. Lett.* 805; Letsinger *et al.* (1988) *J. Am. Chem. Soc.* 110: 4470; and Pauwels *et al.* (1986) *Chemica Scripta* 26: 1419), phosphorothioate (Mag *et al.* (1991) *Nucleic Acids Res.* 19:1437; and U.S. Patent No. 5,644,048), phosphorodithioate (Briu *et al.* (1989) *J. Am. Chem. Soc.* 111 :2321, O-methylphosphoroamidite linkages (*see* Eckstein, *Oligonucleotides and Analogues: A Practical Approach*, Oxford University Press), and peptide nucleic acid backbones and linkages (*see* Egholm (1992) *J. Am. Chem. Soc.* 114:1895; Meier *et al.* (1992) *Chem. Int. Ed. Engl.* 31: 1008; Nielsen (1993) *Nature*, 365: 566; Carlsson *et al.* (1996) *Nature* 380: 207). Other analog nucleic acids include those with positive backbones (Denpcy *et al.* (1995) *Proc. Natl. Acad. Sci. USA* 92: 6097; non-ionic backbones (U.S. Patent Nos. 5,386,023, 5,637,684, 5,602,240, 5,216,141 and 4,469,863; Angew. (1991) *Chem. Intl. Ed. English* 30: 423; Letsinger *et al.* (1988) *J. Am. Chem. Soc.* 110:4470; Letsinger *et al.* (1994) *Nucleoside & Nucleotide* 13:1597; Chapters 2 and 3, ASC Symposium Series 580, "Carbohydrate Modifications in Antisense Research", Ed. Y.S. Sanghui

and P. Dan Cook; Mesmaeker *et al.* (1994), *Bioorganic & Medicinal Chem. Lett.* 4: 395; Jeffs *et al.* (1994) *J. Biomolecular NMR* 34:17; *Tetrahedron Lett.* 37:743 (1996)) and non-ribose backbones, including those described in U.S. Patent Nos. 5,235,033 and 5,034,506, and Chapters 6 and 7, ASC Symposium Series 580, *Carbohydrate Modifications in Antisense Research*, Ed. Y.S. Sanghui and P. Dan Cook. Nucleic acids containing one or more carbocyclic sugars are also included within the definition of nucleic acids (*see Jenkins et al.* (1995), *Chem. Soc. Rev.* pp169-176). Several nucleic acid analogs are described in Rawls, C & E News June 2, 1997 page 35. These modifications of the ribose-phosphate backbone may be done to facilitate the addition of additional moieties such as labels, or to increase the stability and half-life of such molecules in physiological environments.

[0021] The terms "spotting capillary" and "pin" or "printing pin" are used synonymously to refer to the structure that is used to contact a microarray substrate and thereby deposit a reagent to form a microarray feature on that substrate. Unlike many printing pins, however, the spotting capillary is typically a tube and, while not limited to such, in certain preferred embodiments, display a round cross section.

[0022] An "array substrate" refers to the surface or support on which a microarray is printed. Array substrates include, but are not limited to glass, quartz or other minerals, ceramic, porcelain, metal, and metal-coated glass.

[0023] An "array feature" or "array spot" refers to a reagent or reagents deposited at a location on an array surface. Typically a feature is characterized by the presence of one or more specific molecules (*e.g.* particular proteins, nucleic acids, *etc.*).

[0024] A "guide rail" refers to a rail or other device that directs or orients the movement of a platen as described herein. In certain embodiments, guide rail can take any of a number of forms including, but not limited to T-shaped, round, triangular, square, ovoid, and the like. The guide rail is typically coupled to the platen through one or more bearings that permit motion of the platen in one direction (along one axis), but restrict motion in other directions.

[0025] A "print cycle" refers to the sequence of events involved in printing an array feature.

[0026] The term "microarray" refers to an array comprising at least about 10, preferably at least about 50, more preferably at least about 100, still more preferably at least about 500 or 1000, and most preferably at least about 10,000, 40,000, 100,000, or 1,000,000 different and distinct features. Preferred microarrays have an average feature density greater than about 100/cm², more preferably greater than about 1000/cm², still more preferably greater than about 5,000/cm², even still more preferably greater than about 10,000/cm², and most preferably greater than about 20,000/cm², 40,000/cm², 60,000/cm², or even 80,000 cm².

BRIEF DESCRIPTION OF THE DRAWINGS

[0027] Figures 1A and 1B illustrates a microarray printer print head **10** of this invention. Figure 1A illustrates a print head **10** comprising a series of guide plates **14** that support and position the spotting capillaries **12**. The downward motion of the spotting capillaries is limited by a detent **20** and the spotting capillaries are returned to the "extended" position by a spring **18** compressed between the detent and a spring capture plate **22**. The spotting capillaries communicate to a manifold through a flexible capillary tubing **24**. Figure 1B illustrates a print head capable of mounting 64 spotting capillaries on 3 mm centers for 864 well microtiter plates. In this illustration, 16 spotting capillaries are in use.

[0028] Figure 2 illustrates a glass or quartz spotting capillary of this invention showing the inside diameter **32**, the outside diameter **34**, and the bevel **30** at the tip.

[0029] Figure 3 illustrates a two-rail positioning platen compared to the preferred one-rail platen..

[0030] Figure 4 illustrates one embodiment of a platen used to position an array substrate in an array printing device.

[0031] Figure 5 illustrates an exploded view of a platen used to position an array substrate in an array printing device.

[0032] Figure 6 illustrates one embodiment of a platen used to position a microarray print head in an array printing device.

[0033] Figure 7 schematically illustrates vacuum and pressure plumbing of a microarray printer of this invention.

[0034] Figure 8 illustrates a preferred manifold design.

[0035] Figure 9 illustrates the print head and its associated plumbing.

DETAILED DESCRIPTION

[0036] This invention pertains to a microarray printer that can be used to manufacture microarrays (*e.g.* of biochemical samples) by direct contact printing. The array printer of this invention is capable of printing microarrays at higher feature density, with greater speed and lower cost than previous microarray printing devices.

[0037] Without being bound to a particular theory, these efficiencies are achieved by the use of a combination of novel features. A novel printing pin permits vastly more efficient reagent usage and a greater number features to be printed per load. This reduces reagent costs, and because the pin does not require repeated refills during the printing process, the printing operation proceeds more rapidly.

[0038] In addition, positive control of reagent flow using pressure and vacuum, also improves reagent capture and delivery and reduces the incidents of mis-prints due to pin blockage during loads or print steps.

[0039] A novel design for the substrate support permits the use of a larger positioning substrate that can hold a greater number of array substrates (*e.g.* more than 10, preferably more than 20 or 50, still more preferably greater than 100, and most preferably greater than 150, 200, 250, 300, or even greater than about 500 standard slide-sized substrates) and position each array with greater accuracy and precision. The substrate support is kinematically more efficient permitting rapid accelerations and deceleration and thereby permitting a print run to proceed with greater rapidity, *i.e.*, decreasing effective print time and reducing printing costs.

[0040] A related support design for the print head permit rapid acceleration and deceleration of the print head. The rapid positioning of the array substrate combined with the rapid positioning of the print head again, significantly reduces the time required for a print cycle thereby reducing costs.

[0041] These features combine to make possible the efficient printing of microarrays at extremely high efficiency with low reagent usage, and previously unobtainable feature spacing for printed microarrays.

I. Printing pins and the print head.

5 [0042] In one embodiment, this invention provides for print head **10** for printing microarrays and a microarray printer comprising such a print head. As illustrated in Figure 1, in one preferred embodiment, the print head **10** comprises a plurality of spotting capillaries **12** disposed in a support **14** that maintains a fixed spacing between the spotting capillaries and that permits the spotting capillaries to move in a direction parallel to the long axis of said capillaries
10 (*i.e.*, the spotting capillaries can slide in the support).

[0043] The spotting capillaries **12** are preferably cylinders (*e.g.* capillary tubes) made of a rigid material such as glass, quartz or other mineral, ceramic, brittle plastic (*e.g.* acrylic), and the like. It was a surprising discovery of this invention that glass-like materials such as glass or quartz or ceramic could be effectively used as spotting capillaries. Moreover, particularly
15 when fabricated and utilized as described herein, such glass, quartz or ceramic spotting capillaries have a useful lifetime vastly greater than that observed for the commonly utilized metal pins. Indeed, we have yet to determine the maximum lifetime of the spotting capillaries described herein, while it is believed that metal spotting are quite limited in their useful life.

[0044] The spotting capillaries used in this invention can be of any convenient size,
20 however, in preferred embodiments (*see, e.g.*, Figure 2), the spotting capillaries are microcapillaries, with an inside diameter (bore width) of less than about 100 μm , preferably less than about 75 μm , more preferably less than about 50 μm , and most preferably less than about 30 μm , 25 μm or even less than about 20 μm . The load volume (loaded fluid volume) of the spotting capillary is typically 1 μL or less, preferably 0.5 μL or less, more preferably 0.25
25 μL or less, and most preferably 0.2 μL or less or even 0.1 μL or less.

[0045] The spotting capillaries need not have a constant internal diameter. The diameter at the aperture (spotting face) of the spotting capillary will, in part, determine the

minimum feature size of the spotted microarray. Thus, smaller aperture diameters are preferred. Particularly preferred aperture diameters are less than about 75 μ m, more preferably less than about 50 μ m, and most preferably less than about 30 μ m, 25 μ m or even less than about 20 μ m. In certain embodiments, the diameter of the internal channel expands to a maximum of about 75 μ m, preferably about 100 μ m, more preferably about 150 μ m and most preferably about 200 μ m. In one particularly preferred embodiment, illustrated in Figure 2, the spotting capillary has an aperture diameter of about 30 μ m or less and a maximum internal diameter of about 75 μ m or less.

[0046] The spotting capillary outer diameter determines the minimum inter-pin (inter-capillary) spacing, and the inter-pin spacing determines the minimum spacing of the reservoir(s) from which the print head can load reagents. Preferred spotting pins have an outside diameter of about 1 mm or less, more preferably of about 0.7 mm or less, and most preferably of about 0.4 mm or less. The 0.4 mm spotting capillaries can be mounted very close together and, with such close spacing, the print head can load reagents from standard 96 well, 384, well, 864 well, and 1536 well microtiter plates. In preferred embodiments, the center to center spacing of the spotting capillaries is about 10 mm or less, preferably about 5 mm or less, more preferably about 3 mm or less, and most preferably about 2 mm or even 1 mm or less. Such close spotting capillary spacing can be achieved, *e.g.* using the print head designs illustrated herein, allowing the use of even higher density sample reservoirs.

[0047] The support(s) for the spotting capillaries can take any of a number of a number of forms. For example, in one embodiment, the support can comprise a number of channels drilled, etched, or cast in a single metal or plastic piece. The channels then act as guides for the spotting capillaries. Alternatively, the support can be fabricated as by joining a collection of tubes *e.g.* metal tubes. The tubes can be glued or welded together to form a single support structure, each of the tubes acting as a channel for housing a spotting capillary.

[0048] In a particularly preferred embodiment, as illustrated in Figure 1A, the spotting capillaries are supported and positioned by a series of guide plates 14, and optionally, by a guide cylinder 16. To prevent breakage of the spotting capillaries *e.g.*, when they contact irregularities in the substrates, the spotting capillaries are capable of sliding through the guide

plates, *e.g.* when they contact the spotting substrate. The spotting capillaries are then returned to their "extended" position by a spring 18.

[0049] While Figure 1A is illustrated with a coiled spring, it will be appreciated that any of a variety of springs can be used. These include, but are not limited to deformable elastic masses, deformable elastic membranes, "rubber bands", and the like.

[0050] The extended position of the spotting capillaries is limited by a detent 20. The detent can take any convenient form. For example, the detent could comprise a set screw (preferably plastic so as not to damage the spotting capillary), a drop of epoxy or other resin, and the like. In one particularly preferred embodiment, the detent is a disk attached to the spotting capillary. In one embodiment the detent stops up against the pin guide plate. The "downward" extent of the spotting capillary can be determined by the position of attachment of the detent to the spotting capillary. Alternatively, the guide plate can further comprise an adjustment means (*e.g.* a set screw, a shim, *etc.*) for each spotting capillary that can be used to adjust the downward travel for each spotting capillary.

[0051] In certain embodiments, the spring typically rests against a resisting surface, *e.g.* a spring capture plate 22.

[0052] It was also a discovery of this invention that spotting capillaries, particularly when fabricated of glass, quartz, other minerals, or ceramic or porcelain, show a dramatically improved lifetime, when the outer edges of the spotting tip of the spotting capillary, are not flush with the spotting face. Thus, in preferred embodiments, the outer edge of the spotting tip is beveled (*see, e.g.,* 30 in Figure 2).

[0053] The print head typically comprises a plurality of spotting capillaries. Preferred print heads comprise at least two spotting capillaries, more preferably at least 4 spotting capillaries, still more preferably at least 8 or at least 16 spotting capillaries, and most preferably at least 32, 64, 128, or 256 spotting capillaries. Depending on the application, a print head can be configured to use fewer than the total number of available spotting capillaries. Figure 1B illustrates a print head capable of mounting 64 pins on 3 mm centers for 864 well microtiter plates. In this instance, 16 pins are in use.

[0054] The print heads of this invention can be fabricated using standard machining and glass handling techniques well known to those of skill in the art. The spotting capillaries are preferably fabricated by casting or by pulling a quartz or glass microcapillary tube using a commercially available microcapillary puller (*e.g.* Sutter Instrument P-2000 Capillary Puller).

- 5 In particularly preferred embodiments, the microcapillary tip is then beveled using a glass grinder. Such spotting capillaries can be made to order by commercial glass production houses.

II. Platens for array substrate and/or print head positioning.

- [0055] To reliably print an array at high feature density (spots/cm²) it is desirable to reliably and consistently position the spotting capillaries on the microarray substrate. The more
10 precisely and consistently the print head can be positioned relative to the microarray substrate(s), the more possible it becomes to print arrays at a higher feature density.

[0056] Printing a larger number of arrays at a time, however, requires a larger array substrate support (platen). The larger the platen, the more difficult it becomes to reliably and consistently position it relative to print head. One approach to solve this problem is illustrated
15 in Figure 3. The platen illustrated on the left utilizes two guide rails to minimize torque and hysteresis of the platen introduced by the actuator which moves the platen, *e.g.* in the $\pm Y$ direction. To accurately print arrays, it is desirable to accurately position the platen to tolerances better than 50 μm , more preferably better than 25 μm . Such precise positioning requires extremely good bearing alignment for the four bearings and the guide rails must be
20 extremely parallel. The rails and bearings must stay aligned throughout the printing operation. One of skill in the art will appreciate that such a device if will tend to jam and/or introduce positioning imprecision as the actuator drives the platen through jammed positions.

- [0057] Such difficulties are solved with the platens of this invention. One embodiment of a platen 40 of this invention is illustrated by the platen on the right in Figure 3 and in Figure
25 4. This platen utilizes a single guide rail 42 to constrain the position of the support surface 44 that bears the array substrates 56. The support surface communicates with a guide rail 42 through one or more bearings 46. The support surface is coupled to an actuator 48 through a flexible coupling 50.

[0058] The bearing(s) 46 and the guide rail 42 prevent the support surface from rotating in response to a force created by the actuator. However, because there is only a single rail, there are no difficult alignment problems. Straightness of the guide rail is also not critical because any rail deformation will be constant and reproducible, *i.e.*, repeated positioning of the array substrates will be consistent. The platen bears an encoder 58 that accurately encodes the location of the platen along the guide rail.

[0059] The support surface is coupled to the actuator through a flexible coupling 50 that is rigid in the direction of travel ($\pm Y$ direction in Figure 3), but compliant in other directions. This permits the actuator to accurately position the platen along the guide rail while not jamming or binding in other directions. Moreover, it is noted that when the moment arm from the actuator coupling to the array substrate(s) is equal to or preferably greater than the moment arm from the array substrate(s) to the guide rail error introduced at the coupling of the actuator to the support surface will be reduced at the array substrate(s).

[0060] The embodiment illustrated in Figure 4 allows slight motion in the X direction, and yaw, roll and pitch compliance. The system is stiff in the Y direction. One thing to note is that with the flexing, especially pitch, the locations on the platen will vary compared to the encoder, when the encoder is on the motor (actuator) carriage. As long as these variations are reproducible, then the positioning is reliable.

[0061] The flexible coupling 50 illustrated in Figure 4 is a flexible sheet (*e.g.*, sheet metal. This flexure gives freedom in yaw, roll and X, and is stiff in Y, the direction of platen travel. If pitch freedom is desired, it is possible to introduce additional flexible couplings 54. Alternatively, a bearing can be used. The two flexures will 54 be stiff in the Y direction as long as the platen does not lift up under acceleration, and will be stiff in yaw so that all of the yaw compliance will be taken care of in the vertical sheet.

[0062] In certain embodiments, a pivot 52 is provided. In various embodiments, the pivot 52 includes, but is not limited to two points across the width (into the drawing in the side view), a cylinder etc. In this design, pitch motion will require the upper mounting plate to slide across the pivot, so this is preferably lubricated and/or fabricated of low-friction materials, *etc.*

[0063] Figure 5 illustrates one embodiment of an array substrate platen in an exploded view.

[0064] Figure 6 illustrates one embodiment of a platen used to move the print head in a microarray printer of this invention in a $\pm X$ direction (normal to the direction of motion of the array substrate platen). In this embodiment, the platen (support surface) is oriented vertically. A microarray print head **10** is attached to the support surface **44**. In the embodiment illustrated in Figure 5, the actuator **48** is a motor (*e.g.* a linear stepping motor). The actuator **48** is coupled to the support surface through a flexible coupling **50**. The couplings are disposed such that deflection of the support surface is constrained (stiff) in the X direction (the direction of motion), and stiff in the Y direction, but compliant in yaw. The coupling **50a** flexes to relieve Z, yaw, and roll. The platen rides along a truss **58** that bears a guide rail **42**. In a preferred embodiment, the support surface **44** is trapezoidal in shape with the wide side of the trapezoid disposed along the guide rail. This permits the rail bearings to be widely separated and thereby minimize rotation of the support surface. Use of the trapezoidal shape minimizes support surface mass permitting more rapid acceleration and deceleration. The print head **10** is mounted on a Z stage **60** that controls vertical movement of the print head.

[0065] Because the moment arm between the flexible motor coupling **50** and the print head is large compared to the moment arm between the print head and the guide rail **42**, rotations or deflections at the motor coupling have minimal effect on the position of the print head.

[0066] Using the teachings provided herein, one of skill would recognize numerous embodiments for the flexible couplings used in the platens of this invention. In certain embodiments, as indicated above, the flexible couplings comprise flexible sheets (*e.g.* sheets of metal, plastic, or other flexible material). The sheets are selected of materials that are stiff in tension, but capable of bending in other directions. Other flexible couplings include, but are not limited to ball bearings, rod bearings, pin bearings, and the like.

[0067] A wide range of encoders can be used to encode the position of the platen/support surface. Encoders are well known to those of skill in the art and include, but are not limited to optical encoders, mechanical encoders, magnetic encoders, and electronic

encoders. Various electronic encoders include, but are not limited to encoders that convert the change in resistance of a potentiometer or the change in capacitance of a capacitor into a movement or position. Optical encoders include, but are not limited to encoders that convert an optical signal, *e.g.* a bar code, an interferometric measurement, *etc.* into a movement or position. Similarly, magnetic encoders include encoders that a change in magnetic flux or field into a movement or a position. Suitable encoders (*e.g.* with a positional accuracy greater than about 50 μm , preferably with a positional accuracy greater than about 25 μm , more preferably with a positional accuracy greater than about 10 μm , and most preferably with a positional accuracy greater than about 5 μm , greater than about 2 μm , or greater than about 1 μm are commercially available.

[0068] The actuator can be any device or means capable applying a force to the platens of this invention and driving them in a plus or minus direction along the guide rail. Suitable actuators include, but are not limited to stepping motors, linear induction motors, pneumatic actuators, solenoids, piezo-electric actuators, lead screws, and the like. Suitable actuators, and associated motion control products are commercially available from a wide variety of companies (*see, e.g.*, Biorobotics (U.K), Cartesian Technologies Inc. (U.S.A.). Virtek. (Canada), Intelligent Bio-Instruments a division of IAS Products, Inc. (USA), GeneMachines (U.S.A.), Genetix Ltd.(U.K.), Genomic Solutions Inc. (U.S.A.). Genpak Limited. (U.K.), and the like).

[0069] It is noted that in certain preferred embodiments, of the present invention, the slide support platen, with an encoder precision of about 2 μm achieves a spot (array element) precision of about 10 to about 20 μm at any array substrate location on the support surface. particularly preferred platens achieve a spot (array element) precision of better than about 10 μm , more preferably better than about 8 μm and most preferably better than about 5 μm at any array substrate location on the support surface.

[0070] Similarly, the print head positioning platen achieves a precision of about ± 10 μm or less, more preferably about ± 5 μm or less, and most preferably about ± 3 μm or less over the entire slide (array substrate) support surface.

III. Positive and negative pressure control for sample loading and dispensing.

[0071] In a particularly preferred embodiment, the microarray printing devices of this invention utilize positive pressure and negative pressure (vacuum) to control sample loading and dispensing. Each "active" spotting capillary 12 is in fluid communication, *e.g.* via capillary tubing 70 with a manifold 64 (*see, e.g.*, Figures 7, 8, and 9) that permits the application of pressure or vacuum to the spotting capillaries.

[0072] A preferred plumbing scheme is illustrated in Figure 7. In preferred embodiments, the gas flow (*e.g.* air, nitrogen, argon, *etc.*) flows through the manifold always in the same direction for all operations. This assures that any liquid drops that may be left in the tubing always will be forced to move toward the waste bottle and will not be blown back into the manifold. If droplets do get into the manifold they may block the supply of printing pressure to one or more pins, thus reducing the reliability of the printing.

[0073] In general, the plumbing system comprises a pressure source 78, and a vacuum source 80. The pressure and vacuum sources are in fluid communication with a manifold 74, *e.g.* via gas and fluid tubing 84. The manifold is also in fluid communication with the spotting capillaries so that pressure or vacuum applied to the manifold is delivered to the channel (bore) in the capillaries. In preferred embodiments, there is a waste receptacle disposed between the low pressure/vacuum source 80 and the manifold 74.

[0074] In preferred embodiments, the system is designed so that the tubing from the waste bottle to the manifold preferably slopes downward to facilitate liquid flow, and we strive to minimize the volume of the tubing and waste receptacle so that the pressure changes are transmitted to the manifold quickly. Thus, while the tubing communicating the pressure and vacuum to the manifold and communicating the manifold to the waste receptacle can be essentially any convenient tubing (as long as it is resistant to the reagents employed), in preferred embodiments, the tubing is a low void volume tubing (*e.g.* a fine bore capillary tubing). Such tubings are well known to those of skill in the art.

[0075] In one preferred embodiment the pressure source 78 can apply two pressures, a blowout pressure (*e.g.* 15 PSI), and a positive pressure used during printing (*e.g.* from about 0.1 to 2, preferably from about 0.1 to 1, more preferably from about 0.1 to about 0.5, and most

preferably about 0.3 inches of water). Similarly, in preferred embodiments, the vacuum source **80**, can apply two pressures, a "high vacuum" for cleaning, and a "low vacuum" for printing operations. The waste receptacle is preferably a low volume waste receptacle (*e.g.* a 200 ml waste bottle).

5 **[0076]** A preferred manifold is illustrated in Figure 8. This manifold comprises a common channel with an inlet port (manifold inlet) **66** and an outlet port (manifold outlet) **68**. In fluid communication with the manifold are a number of capillary connectors **76** that each with an internal (manifold) capillary port **72**, and an external capillary port **68**. Each capillary connector is disposed to receive a connection (*e.g.* a tubing connection) to provide a fluid
10 communication to a spotting capillary **12**. The internal capillary port **72** is disposed inwards into the manifold so that the capillary port is not flush with the internal wall of the manifold. This prevents droplets from accumulating on the internal capillary port **72** which could interfere with reliable loading or delivery of samples.

15 **[0077]** The manifold can be made of any of a variety of materials and can take a number of different shapes. Preferred shapes however, permit the rapid distribution of pressure, permit the unidirectional flow of gas and waste, and permit the disposition of the internal ports **72** away from the internal surface of the manifold. Useful materials, include various plastics, glass, quartz, ceramic, and metals. In one preferred embodiment, the manifold is fabricated from stainless steel.

20 **[0078]** A plumbed print head is illustrated in Figure 9. This figure illustrates the print head **10** comprising a plurality of spotting capillaries **12** (four visible in the figure). The spotting capillaries are in fluid communication with the manifold **64** via flexible capillary tubing **70**. A pressure line **84a** can be seen at the upper right and a waste/vacuum line **84b** can be seen at the upper left.

25 **IV. Preparation of a microarray.**

[0079] The microarray printer of this invention can be used to print microarrays comprising essentially any molecules that can be suspended, dissolved, or otherwise placed in a solution. Preferred microarrays include, but are not limited to microarrays of biomolecules (*e.g.* sugars, carbohydrates, nucleic acid, proteins, and the like). Particularly preferred

microarrays include nucleic acid and/or protein arrays. Methods of preparing and/or purifying biomolecules are well known to those of skill in the art (*see, e.g.*, Berger and Kimmel (1989) *Guide to Molecular Cloning Techniques*, Methods in Enzymology 152 Academic Press, Inc., San Diego, CA; Sambrook *et al.* (1989) *Molecular Cloning- A Laboratory Manual* (2nd ed.) Vol. 1-3, Cold Spring Harbor Laboratory, Cold Spring Harbor Press, NY; Ausubel *et al.* (1994) *Current Protocols in Molecular Biology*, Current Protocols, a joint venture between Greene Publishing Associates, Inc. and John Wiley & Sons, Inc., (1994 Supplement) (Ausubel); U.S. Patent 5,017,478; and European Patent No. 0,246,864.).

[0080] The materials that are to be printed (*e.g.* proteins, nucleic acids, *etc.*) are typically formulated in "printing solutions". Solutions for microarray printing are well known to those of skill in the art (*see, e.g.*, U.S. Patents 6,101,946, 5,958,342; and MacBeath and Schreiber (2000) *Science* 289: 1760-1763; Mark Schena (Ed.) (1999) *Genes, Genomes and Chips. In DNA Microarrays: A Practical Approach*, Oxford University Press, Oxford).

[0081] The microarrays can be fabricated on any of a wide variety of substrates well known to those of skill in the art. Such substrates include, but are not limited to glass, plastic, quartz and other minerals, metal, ceramic, porcelain, metal covered (*e.g.* sputtered) glass, and the like. A number of substrates, often derivatized to facilitate microarray printing are commercially available (*see, e.g.*, silane slides from Sigma Chemical Co., the SuperCleanTM, SuperAmideTM, and SuperAldehydeTM substrates from Telechem, International Inc., *etc.*).

[0082] In operation, the printing pins (spotting capillaries) are initially washed. In a preferred embodiment, this involves moving the print head over a wash bath and applying pressure (*e.g.*, about 15 PSI) to the manifold to blow out any liquid remaining in the spotting capillaries. The spotting capillaries are dipped in and out of a cleaning solution (*e.g.* 0.001% Micro-90, Cole Palmer Inc.), in a sonicating bath at about 0.75 sec intervals for about 3 cycles while pressurized.

[0083] The device is switched from pressure to house ("high") vacuum and the spotting capillaries are dipped into the sonicated cleaning solution for 3 more cycles while drawing cleaning solution into the spotting capillaries. The spotting capillaries are then moved to a sonicating rinse bath that contains pure (*e.g.* double distilled) water.

[0084] The spotting capillaries are dipped in and out of water for about 3 cycles of about 0.75 sec each. This allows the formation of interspersed air bubbles and water in the tubing, assuring that the cleaning solution is more effectively removed from the walls of the tubes. Finally the spotting capillaries are dried by sucking air through them using house vacuum, blowing hot air over the spotting capillaries for about 4 sec, opening a vent to atmosphere and continuing vacuum for about 2 sec in order to clear liquid from the manifold and waste tubing (see Figure 7).

[0085] The spotting capillaries are then filled by dipping the spotting capillaries into reagent reservoir(s) (e.g. a microtiter plate) containing the printing solution(s). Full house vacuum is applied to the manifold for about 0.15 sec in order to assure that the solutions enter the tips of the pins. The manifold is then vented to atmospheric pressure and the spotting capillaries are allowed to sit in the printing solutions for about an additional 0.75 sec so that capillary action fills the spotting capillaries. In one preferred embodiment, the spotting capillaries each contain approximately 0.2 ml when full. The spotting capillaries do not fill beyond their tops due to capillary action – the solutions do not enter the flexible tubing that connects the pins to the manifold. In some cases a slight vacuum of ~ 0.2 inches of water is used to assist the filling. This is adjusted to be low enough so that the no liquid is drawn beyond the top of the pins. The entire wash, dry and fill functions take about 25 seconds.

[0086] To print an array feature or features, the print head is moved over the first printing substrate and lowered to make contact, and raised. In one preferred embodiment, in the upper position the tips of the printing pins are about 0.5 to 1.0 mm above the array substrate, and when printing the print head is lowered so that the pins would move about 0.2 mm below the substrate surface if no array substrate were present. When a substrate is present the spotting capillary tips contact it and the spring mounts allow the spotting capillaries to stop moving while the print head body continues its motion toward the substrate. In a preferred embodiment, the total time for the print head to move down, contact the slide and return to the upper position is about 0.05 to 0.2 sec. During the printing operation a constant pressure of 0.1 to 0.4 inches of water is applied to the manifold to keep the printing solutions at the tips of the spotting capillaries. This assures that the spotting capillaries are wet with the printing solutions so that liquid will be transferred to the substrate on contact. The pressure does not eject the

printing solution. If this is not done, the solutions can pull away from the tips and the printing will stop. The small diameter of the tubing and the printing pins provides enough flow resistance to air so that the manifold pressure is maintained even if one or more of the pins does not contain printing solution. The cycle is repeated to print additional features or other array substrates.

[0087] The amount of printing solution that is deposited on the substrate depends on the interaction between the substrate and the printing solution, and the diameter of the tip of the printing pin. When printing salt solutions on glass, each fill of the printing pin (0.2 ml) can make at least 10,000 spots. When printing 20% DMSO solutions with DNA, the same load can print at least 2000 spots. This is many more spots than are possible with other printing systems. Thus the system described above can deposit less than 100 pL per spot in typical printing.

[0088] Use of the novel spotting capillaries and/or print heads of this invention provides extremely efficient reagent usage. In certain embodiments, the printer can print at least about 500 spots (features) per 0.2 μ L load, more preferably at least about 1000 spots (features) per 0.2 μ L load, most preferably at least about 1500 spots (features) per 0.2 μ L load, at least about 2000 spots (features) per 0.2 μ L load, at least about 5,000 spots (features) per 0.2 μ L load, or at least about 10,000 spots (features) per 0.2 μ L load. Because the printing capacities are so high, the print head typically does not need to refill during a print run. This greatly decreases the duration of a print run.

V. Microarray printing device.

[0089] The various elements described above, *e.g.* spotting capillaries, print head design, array support platen, print head support platen, vacuum and pressure system, manifold, sample loading unloading protocols, and the like can be incorporated individually, or in combination, into preexisting microarray printers or they can be assembled into a microarray printer built *de novo*.

[0090] Methods of designing and building microarray printing devices are generally known to those of skill in the art (*see, e.g.*, U.S. Patents 6,110,426, and 5,807,522, and

publications of the Brown Laboratory at Stanford University (*e.g.*, *The McGuide. Version 2.0*, available on the internet at <http://cmgm.stanford.edu/pbrown/mguide/index.html>, and from Cold Spring Harbor Laboratories).

[0091] In general, microarray printers of this invention will, in preferred embodiments, include a base adapted to hold reservoir(s) of printing solutions, a platen for supporting and positioning microarray substrates, and a platen for supporting and positioning a print head. The microarray printer will include actuators (*e.g.* motors) for driving/positioning the various platens and for vertically positioning the print head. The microarray printer, will typically include associated electronics to read encoded platen positions and/or to drive the various actuators to position the array substrates and print head. Typically such electronics will include a computer controller. The microarray printer can additionally comprise vacuum and pressure lines, reagent reservoirs, waste receptacles, cleaning baths and the like as described herein.

[0092] In a particularly preferred embodiment, the microarray printer will include the print head platen, the array substrate platen, a print head comprising spotting capillaries as described herein. The microarray printer will also preferably also include pressure and vacuum sources as described herein. While it is preferred that the microarray printer comprise all of the elements described herein, it is not required that all such elements be present. Thus, in certain embodiments, the microarray printer comprises one, two, or only a few of such elements. Thus, for example certain microarray printers may only comprise a print head according to this invention and/or the array substrate platen, and/or the print head support platen, and so forth.

VI. Microarrays.

[0093] It is believed that the microarray printers of this invention permit the production of spotted microarrays with an accuracy, consistency, and array feature density previously unavailable. Thus, in certain embodiments, this invention provides high-density microarrays comprising a plurality of molecules, preferably biomolecules where the array comprises at least about 1,000 features (spots), preferably at least about 10,000 features (spots), more preferably at least about 40,000 features (spots), and most preferably at least about 100,000 features (spots), or at least about 1,000,000 features (spots). In particularly preferred embodiments, the features are present at an average center-to-center spacing of about 130 μm or less, preferably

about 100 μm or less, more preferably about 80 μm or less, and most preferably about 65, 50, or 40 μm or less.

[0094] In preferred embodiments, the microarray is a protein and/or nucleic acid microarray. In nucleic acid arrays the nucleic acids preferably have an average length greater than about 100 nucleotides, preferably greater than about 500 nucleotides, more preferably greater than about 1,000 nucleotides, and most preferably greater than about 2,000, 3,000, 5,000, or even 10,000 nucleotides.

[0095] Preferred arrays of this invention have a feature (spot) density greater than about 20,000 features/ cm^2 , preferably greater than about 30,000 features/ cm^2 , more preferably greater than about 40,000 features/ cm^2 , and most preferably greater than about greater than about 50,000 or 60,000 features/ cm^2 .

[0096] Because the microarrays are spotted, the reagents are typically simply spotted, in preferred embodiments, the molecule(s) comprising the array features are simply adsorbed to said substrate. However, in certain embodiments the reagents and/or the array substrate can be derivatized so that the molecules comprising the features (spots) covalently couple to the substrate. Methods of so derivatizing macromolecules are well known to those of skill in the art. Thus, for example, the reagents can be derivatized with a sulfhydryl group (-SH) which will covalently couple to a gold surface (*e.g.* gold coated glass).

V. Kits.

[0097] In still another embodiment, this invention provides kits comprising one or more containers containing the arrays described above. In certain embodiments, the arrays will comprise features representing nucleic acids from every chromosome in a subject organism (*e.g.* a human). In certain embodiments, the arrays will comprise features representing nucleic acids from every known expressed sequence tag (EST) for a given organism, or tissue, or whose expression is associated with a particular physiological state (*e.g.* a particular pathology).

[0098] These array constituents are merely illustrative. Numerous other arrays components will be recognized by one of ordinary skill in the art.



[0100] It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be

[illegible]